

Attenuation of experimental cataract by vitamin C in rabbits

Seyedeh Zeinab Peighambarzadeh*, Mehdi Tavana

¹Department of Veterinary Medicine, Faculty of Agriculture, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

Abstract: Development of a drug which could prevent or delay the onset or progression of cataract will help to reduce the number of people getting blind due to cataract worldwide. This study was undertaken to evaluate the clinical and biochemical changes of the crystalline lens and gel-electrophoresis of water soluble proteins in a selenite-induced cataract and to assess the preventive role of vitamin C in rabbit as an animal model. Selenite cataract was produced in rabbits by subcutaneous administration of sodium selenite. Biochemical and clinical changes following induction of selenite cataract in White newzland rabbits were studied the role of vitamin C in attenuating or preventing cataract genesis. Vitamin C was capable of preventing selenite cataractogenesis. Selenite cataract did not develop in 40% vitamin C treated rabbits, when administered on the 10th and 24th post-partum day respectively. The study confirmed that selenite induced cataract in White newzland rabbits is attenuated by vitamin C.

Key words: *Cataract; Vitamin C; Rabbits*

1. Introduction

Cataract is the most common cause of blindness worldwide and its incidence will increase as the world's population ages (80). Looking for preventive methods to delay the onset of cataract can help to narrow the gap between the incidence of cataract blindness and the provision of surgical treatment. Selenite-induced cataract is an extremely rapid and convenient model for simulating senile nuclear cataract. It is generally accepted that oxidative insult plays a key role in the opacification process, and redox cycles are important for the maintenance of lens transparency. Free radical-induced oxidative stress has also been found to be one of the major triggering factors for senile cataract formation. The selenite cataract model is an appropriate specimen for the study of cataractogenesis, since it exhibits many of the morphological changes observed in senile cataract. Rabbits of different species and from different origins, display varied susceptibility to formation of selenite cataract. Devamanoharan et al reported 15% cataract on the 15th day of experiment after 0.3 mmol ascorbate and selenite treatment in White newzland rabbits, while Orhan et al observed 41.6% cataract on the same day of the experiment in Wistar albino rabbits. Administration of selenite increases peroxidation of lens lipids as well as formation of H_2O_2 in the aqueous humour. This disturbance of oxidative stress is corrected by various antioxidants. These include sodium ascorbate (Vitamin C(4); Pantethine, Glutathione isopropyl ester, Glutathione, L-ascorbic acid phosphate, Cysteamine S-Phosphate (Hiraoka et

al., 1996); WR - 77913, an amino phosphorothioate (Clark and Steele, 1992), Deferoxamine (Varma et al., 1982); Propolis, Diclofenac, Vitamin C, Quercetin; Naproxen (Gupta and Joshi, 1994); and S-diethylsuccinyl glutathione isopropyl ester (Blunt and Takemoto, 2000). Vitamin-E has been found to inhibit lipid peroxidation and cataract formation in organ culture (Tappel et al., 1975) in experimental animal models *in vivo* (Simon and Hudes, 1999) and in corticosteroid-induced cataract (Kojima et al., 1996). But, the antioxidant property of vitamin C has not been examined in selenite cataract. The present study was designed to test the anti-cataractogenic property of Vitamin C in selenite cataract (Mathew et al., 1996; Mathew, 1998; Mathew et al., 1998).

A significant number of epidemiological studies have been published regarding the potential role of antioxidants in the prevention of cataract.¹⁰⁻¹⁸ Although the majority of these studies have shown a positive correlation between higher dietary antioxidant intake and decreased cataract formation (Orhan et al 1999, Varma, 1991), conflicting results exist between different epidemiological studies (Leske et al., 1998, Varma, 1991).

2. Material and methods

Frothy White newzland rabbit pups were provided by the Laboratory Animal Research Center of Shiraz University of Medical Sciences. Animal handling, maintenance and experimentations were done in accordance with the guidelines set by the Institutional Animal Ethical Committee. The animal room was well-ventilated and had a regular 12:12-h light/dark cycle throughout the experimental period. Rabbits were divided into three groups: two

* Corresponding Author.

experimental and one control. In group 1, 100 µg of saline was injected subcutaneously on postpartum day 10. In the two experimental groups (2-3), 100 µl of sodium selenite solution (20 µmol/kg of dissolved solution in distilled water) was injected subcutaneously on postpartum day 10. In addition, rabbits in group 3 received subcutaneous injections of 2.50mg vitamin C (vit c)/rabbit pups on postpartum day 8 (2 days prior to the selenite injection) and this injection was repeated once daily for 14 and 28 consecutive days thereafter in groups 3 respectively.

Development of cataract in the rabbits' eyes was assessed every other day for 2 weeks after selenite injection using slit-lamp biomicroscopy by an ophthalmologist. Prior to each examination, mydriasis was achieved in each eye by instilling one drop of a 0.5% tropic amide every 10 min for 3 times. The eyes were viewed under a slit-lamp biomicroscope at 16× magnification; the observer was blind to the group of the animals before classifying the cataracts. Any cataracts developed were classified as below and photographed. Lens opacification was classified into no opacification (clear lens), subcapsular cataract, nuclear cataract and mature cataract (lens with a dense opacity involving the entire lens).

Following the final examinations, the animals in subgroups 1a, 2a, 3a were sacrificed by high dose inhalation of ether on day 14 after administration of selenite while the subgroups 1b, 2b, 3b were euthanized on the 28th day. Enucleation was done and the lenses were dissected out for various biochemical and electrophoretic studies.

The lenses from each group of rabbits were homogenized in 10 mM phosphate buffer (pH=8), and centrifuged at 3500 rpm for 3 min at 4°C. The supernatant obtained was stored at -20°C; being used for the analysis of total and soluble protein concentration, and SDS-PAGE. The total protein content of the samples was determined by the method of Lowry et al.¹⁹ using bovine serum albumin as the standard. Soluble protein concentration was measured spectrophotometrically. The mentioned supernatants were diluted with distilled water to achieve 1/20 dilution and the absorbences were recorded at 280 and 260 nanometer and then protein concentration was measured using the following formula: mg protein/ml=(A280X1.55)-(A260X0.76).

SDS slab polyacrylamide gel electrophoresis was conducted according to the Laemmli's method (1970); using 12.5% acrylamide separating gel.²⁰ Protein samples were added to the loading buffer to give a final concentration of 1 mg/ml protein, 0.01 mol/ml Tris-HCl, pH=6.8, 0.4% SDS, 100 g/l glycerol and 0.04 g/l bromophenol blue. The running gel with dimensions of 140×140×1 mm was made of 50-200 g/l gradient polyacrylamide in 1.2 mol/l Tris-HCl, pH=8.8 and 3 g/l SDS. The stacking gel contained 30 g/l acrylamide in 0.25 mol/l Tris-HCl, pH=6.8 and 2 g/l SDS. The electrode buffer was comprised of 0.025 mol/l Tris-HCl, 0.192 mol/l glycine and 0.15 % SDS at pH=8.16. Electrophoresis was performed at a constant 25 mA current and the gel was stained with 0.25 % Coomassie Brilliant Blue R-250 in 50% acetic acid/25% methanol and de-stained with 10% acetic acid/7% methanol.

All statistical calculations were carried out with the SPSS software (version 11.5, Chicago, IL, USA). The values are expressed as the mean±SD. The data were statistically analyzed; using Chi-Square test to evaluate differences in cataract pattern among all 5 groups, one way ANOVA (comparison of total and soluble proteins among 5 groups) and two way ANOVA (comparison of the effect of days of sampling, i.e. 14 and 28). There was a significant difference between means; using Duncan's multiple range test at the level of p<0.05.

3. Results

Table 1 shows the effect of different reagents on cataract formation in rabbits of 3 groups. On postpartum day 24th (2 weeks after selenite injection), the rabbits were evaluated for cataract development. None of the rabbits in the control group (group 1, no selenite) had cataract. In group 2, all of the rabbits (100%) had developed cataracts classified as subcapsular, nuclear and mature cataract (lens with a complete dense opacity involving the entire lens). However, in group 3, which had received vitamin c injections in addition to selenite, only 24 of 40 (60%) eyes developed cataract (mature cataract in 16 rabbits and nuclear cataract in 8 rabbits). This difference (between groups 3 and 2) was statistically significant (P=0.001). The remaining 16 of 40 (40%) eyes of group 3 had clear, normal lenses.

Table 1: The effect of different reagents on cataract formation in rabbits

Groups	Intervention	No. of eyes	Examination at 24 days old
Group1	Control(saline)	40	Clear lens
Group2	Selenite sodium	40	29 mature cataract 8 nuclear cataract 5 posterior sub capsular cataract (PSCC)
Group3	Selenite and vitamin C	40	14 mature cataract 10 nuclear cataract 16 clear lens

Table 2 shows the effects of different treatments on the proteins of the lens. After 14 and 28 days

post-selenite injection, the total lens protein concentration (expressed as mg protein/ml lens

extract) was significantly ($P=0.050$) lower than those in group 2 (selenite alone) as compared with group 1 (control), group 3 ((selenite+vitamin C). After 14 days, the mean concentrations of soluble protein in the lenses of group 2a rabbits were significantly ($P=0.050$) lower than those of groups 1a, 3a rabbits.

After 28 days, the mean concentrations of soluble protein in the lenses of group 2b rabbits were significantly ($P=0.050$) lower than those of group 1b rabbits but not significantly different from groups 3b rabbits ($P>0.050$).

Table 2: Mean and \pm SD of total and soluble protein content of lenses of rabbits treated by different reagents

Groups	Days after selenite injection	No. of eyes	Total protein (mg/ml)	Soluble protein (mg/ml)
1a	14	18	432.00 \pm 254.00	432.5 \pm 65.5
1b	28	22	890.00 \pm 187.50	653.8 \pm 215.6
2a*	14	16	352.00 \pm 154.50	178.5 \pm 90.50
2b**	28	24	154.00 \pm 67.00	256.6 \pm 125.2
3a	14	16	702.50 \pm 218.00	387.50 \pm 97.10
3b	28	24	493.00 \pm 208.00	198.4 \pm 124.5

*Values of group 2a were significantly different from those of group 1a and 3a ($P<0.050$).

**Values of group 2b were significantly different from those of group 1b ($P<0.050$).

The SDS-PAGE profile of the lens proteins was carried out to visualize the effect of sodium selenite and prophylaxis with vitamin E. Group 1 (a and b) showed the normal protein profile. In group 2 (a and b), two bands were detected which had a lower intensity than that of the control group and corresponded to proteins with 18 kDa to 32 kDa and more than 45 kDa molecular weights. These bands showed near to normal expression in groups 3 (a and b), as found in group 1.

4. Discussion

As the world's population ages, cataract-induced visual dysfunction and blindness are on the increase. Cataract is a major cause of blindness and of severe visual impairment leading to bilateral blindness in an estimated 20 million people worldwide. In developing countries, 50–90% of all blindness is caused by cataracts. Pharmacological treatment against human cataract has so far not been achieved. Therefore, surgery to remove the opacified lens is the only effective treatment for the cataract. The challenges are to prevent or delay cataract formation and also to treat eventually if it occurs. The exact mechanism of cataract formation is still not very clear. There are studies to investigate the mechanism of cataractogenesis using different models

Of cataract and to target critical steps to stop this process. Among various models, the selenite cataract model is one of the most commonly used experimental models. Selenite-induced cataract has been proven to be a rapid and convenient animal model for cataract (Tappel et al., 1975; Varma, 1991). Several biochemical processes such as oxidative stress, altered epithelial metabolism, calcium accumulation, crystalline precipitation and cytoskeletal loss occur during the development of selenite-induced cataract (Ohta et al., 1996; Seddon et al., 1994).

Selenite cataract is initiated by the oxidation of the lens membrane sulfhydryl (SH) groups by the action of selenite. This mainly inactivates the Ca-ATPases and alters the ion homeostasis. The influx of calcium activates protease, Calpain II, which partially

degrades lens-specific proteins and β -crystallins. These partially degraded crystallins expose the hydrophobic regions and the thiol groups, which are then oxidised by selenite (Seddon et al., 1994) or by ROS and lipid peroxides generated in the lens, to form insoluble proteins and cause opaqueness. Besides this, ROS that penetrates across the aqueous humour from other tissue sites enhances the process (Mathew, 1998). Lipid peroxides ultimately resolve into malondialdehyde (MDA) as the end product (Shearer et al., 1992). It is possible that the carbamyl group of MDA could react with primary amino groups of protein and phospholipids of lenticular plasmalemmae by a crosslinking reaction forming schiff-base conjugate. Such a cross linking mechanism could initiate in the lipid bilayer of ocular cell membranes even by a small amount of MDA. Lipid peroxides can also oxidise SH groups of proteins by itself. The oxidation of the SH groups of proteins further undergo disulphide cross linking, forming high molecular weight aggregates leading to the maturation of cataract.

In the study, all (100%) of the rabbits receiving a single subcutaneous injection of sodium selenite (20 μ mol/kg) on postpartum day 10th developed cataracts, changes in protein profiles of the lens. In addition, SDS-PAGE results showed changes in soluble proteins profiles.

The well-established concept that intraocular generation of the oxygen-free radicals initiate oxidative stress, resulting in cataract formation, was initially proposed by many laboratories,²⁴⁻²⁶ and the role of oxidative stress in cataract development and the importance of antioxidants in prevention of cataract has been a subject of research in ophthalmology but there have been conflicting results (Orhan et al 1999; Spector and Garner, 1981; Varma, 1991).

In this study, we have focused on vitamin C in preventing cataract and its associated biochemical and electrophoretic changes. Based on the results, subcutaneous injections of 2.50mg vitamin C prevented selenite-induced cataract in 40% of rabbits. Vitamin C group had significantly higher total lens protein and soluble protein

concentrations compared with the group of rabbits that only received sodium selenite. In addition, vitamin C group showed normal appearance of the bands of soluble lens proteins in gel electrophoresis.

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